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Cloning of the chicken integrin α4 gene

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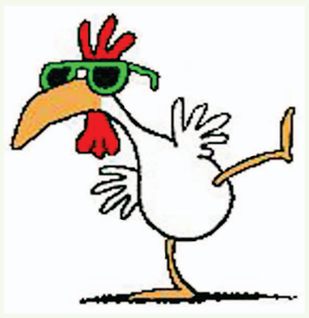
Conclusion
We have shown that mRNA encoding the integrin α4 protein is expressed in the chicken, and for the first time, the integrin α4 cDNA has been cloned. The sequence reveals a polypeptide of 1021 amino acids with homology to the known mammalian integrin α4 proteins. Structurally, chicken integrin α4 resembles the mammalian counterparts; however, further investigations are necessary to determine whether chicken integrin α4 is involved in leukocyte homing.



Background

Integrins are heterodimeric membrane-spanning proteins composed of an α and a β chain, and they function as cell adhesion receptors that recognize extracellular matrix ligands and cell-surface ligands. In mammals, integrin α4 dimerizes with either integrin β1 or β7. Both dimers can be expressed on leukocytes and are involved in leukocyte homing to different tissues, where the integrin dimers interact with distinct receptors on endothelial cells (see Fig. 1). This interaction contributes to the rolling, adhesion, and extravasation of leukocytes.
In chickens, the existence of the integrin α4 gene has been predicted from computational analysis of the genomic sequence, and the finding of several expressed sequence tags supports the notion that the gene is transcribed into mRNA. However, the gene has not yet been cloned.

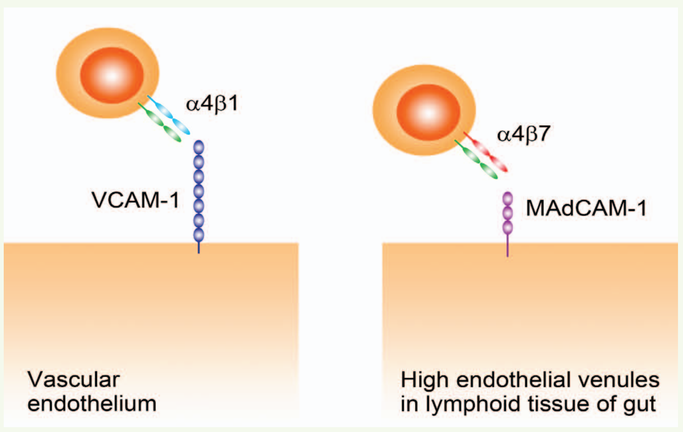


Figure 1. In mammals, integrin α4β1 dimers bind to Vascular Cell Adhesion Molecule-1 (VCAM-1), which is expressed on vascular endothelium throughout the circulatory system. Integrin α4β7 dimers bind to Mucosal Addressin Cell Adhesion Molecule-1 (MAdCAM-1), which is restricted to endothelial cells in lymphoid tissues of the gut, such as Peyer's patches.

Discussion

In mammals, integrin α4 forms dimers with integrin β1 or β7. Since the predicted protein sequence and structure of chicken integrin α4 is similar to the known mammalian orthologs, one could assume similar functional properties. However, searches in the chicken genomic database only reveal the integrin β1 gene and not the integrin β7 gene. The integrin α4β7 dimer is responsible for leukocyte homing to lymphoid tissue of the gut. What the lack of integrin β7 in the chicken means for leukocyte homing is not known. Perhaps another member of the integrin β family takes over or perhaps other homing mechanisms are in play.

Results

The chicken integrin α4 gene

According to the Ensembl database, the chicken integrin α4 gene is located on chromosome 7 and consists of 28 exons (Fig. 2). The gene spans 32.3 kb.

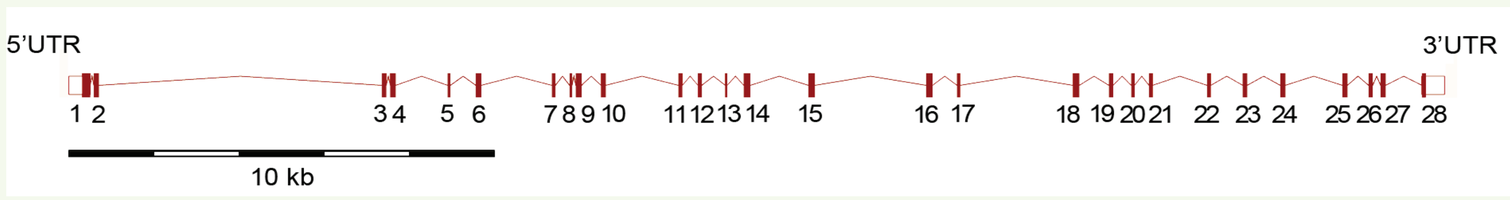


Figure 2. Genomic organization of the gene encoding chicken integrin α4. The gene consists of 28 exons that span 32.3 kb on chromosome 7. Data was obtained from Ensembl Genome Browser (http://www.ensembl.org/index.html).

Cloning of integrin α4 cDNA

Total RNA was purified from chicken peripheral blood leukocytes and converted into cDNA, which was used as template in PCR amplification. The integrin α4 cDNA was successfully cloned using primers located in the 5' and 3' untranslated regions. The cDNA sequence contains 3755 nucleotides including an open reading frame encoding a polypeptide of 1021 amino acids. Two nucleotides in the open reading frame (T321 and A567) differ from the database sequence (Ensembl transcript ENSGALT0000001460, UniProtKB 7Q9PSD7_CHICK), but in both cases the nucleotide substitution does not change the encoded amino acid. The amino acid sequence is shown in Fig. 3. The calculated molecular mass is 114 kDa.

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MRSCGRAARW AAPLLLLWQC LPTARTYNVD TRHPLLFRGD NGTFFGYSVL LMGHGEERWL   60
VVGAPQASWA ANSSVISPGA IFRCRIGSNP RGSCEQLQLG HPSGEYCGKT CMEERDYQWL  120
GVSLSRQPRE NGSFVACGHR WKNIFYIKND HKLPHGICFA VSSDFRTELS RRICPCYKDH  180
VRKFGENHGS CQAGMSSFYI GDLIIMGAPG SYYWTGSVFV YNTTINTIHA YTHSNNQVKF  240
GSYLGYSVGA GHFLTPSSTE VIGGAPQQEQ TGKAFIFSID EHLNVLFEVK GKKLGSYFGA  300
SVCAVDLNSD GLSDLLVGAP MESTIREEGR VYVYINSGSK AEMVELDIEL SGSDSYAARF  360
GESITNLGDI DNDGFEDVAI GAPQEDDLKG AIYIYNGRED GITPSFSQRI PAQQVSTSLS  420
MFGQSIASGI DADNNGYQDI AVGAFLSDSA VVLRTKPVII VEAFLKHPKS INRTNLNCME  480
NDQPAICVNL QICFNYTGQG VPDNTEMFYN LSVDVKRRVD TQARFYFSAN GTSETTSGSI  540
KINRKIIACK GHLAFMRKDV RDILTPVHVE ASYHLGQQIL QKRDNQELSA LPPVLQRRKE  600
KDIIKSKFVF ARICSQENCS ADLRVSGKVA FPKPHDKKMY LVVGSTKTLL LNISLHNAGD  660
DAYETVLHIQ FPKGLYFIRV PDLEEKQIHC EVLDKDIHAV KLECSVGYLY VDQNSKLDLS  720
FPLDTSSFTR AEDDLNIIIN VSCKNENENL LLDNMVTVAV PLKYETELNT HGFVTPPSFV  780
YGTNENEASV MCMEENINFT FHVINAGPSM APNINLELMI PNAFPPHDFK LFNVMDIKTT  840
VGECSYNEYP RNCNAPEKTE NILKDVVTFF SKPAKRQMYC MKNDSLCLQI HCKLGNMENG  900
KEATIQLHLE ATPALLEMDD ASTLKFEVRA TASPEKNAKV IELQKDKQVA YVYLEGVHHQ  960
KPKYHVTVLI IGIGLIAGIT LFLLLSLLLW KIGFFKRQYK PIPQDMNRRE SWSFTSGNKD 1020
D                                                                   1021
```

Figure 3. Amino acid sequence of chicken integrin α4.

Sequence comparison

The amino acid sequence of chicken integrin α4 is similar to integrin α4 sequences in other species. Multiple alignments using the ClustalW2 alignment tool at http://www.ebi.ac.uk demonstrate a sequence identity of 52-61% between chicken integrin α4 and the integrin α4 of several mammals (Table I). Between the mammalian species, the sequence identity is higher (70-99%).

Table I. Sequence identity in percent between integrin α4 proteins of different species.

Identity (%)	Chicken	Human	Chimp-anzee	Mouse	Rat	Dog	Cow
Chicken	-	60.7	60.7	60.4	59.8	52.3	61.0
Human	60.7	-	99.4	84.8	84.1	74.1	85.6
Chimp-anzee	60.7	99.4	-	85.0	84.2	74.3	85.8
Mouse	60.4	84.8	85.0	-	93.8	70.5	80.4
Rat	59.8	84.1	84.2	93.8	-	69.8	79.8
Dog	52.3	74.1	74.3	70.5	69.8	-	71.7
Cow	61.0	85.6	85.8	80.4	79.8	71.7	-

Protein structure

The integrin α4 protein has a large extracellular region consisting of aa 1-967 (of which the first 26 aa constitute a signal peptide), and this is followed by a short transmembrane region (aa 968-990) and a cytoplasmic tail (aa 991-1021). As shown in Fig. 4, the domain architecture is similar to that of integrin α4 in mammals, and the extracellular region includes the so-called β-propellor, thigh, calf1, and calf2 domains.

Figure 4. Protein structure of chicken integrin α4 as predicted by InterProScan Sequence Search (http://www.ebi.ac.uk/Tools/-InterProScan/). SIG: signal peptide, TM: transmembrane region, CYT: cytoplasmic tail.